REGENERATION OF CERTAIN STRUCTURES IN FUNDULUS HETEROCLITUS.

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It is a fact of common knowledge to all observers of fish kept in aquaria, that portions of the fins, lost through accident, or purposely removed will be replaced by new structures which cannot be distinguished, in many cases, from normal fins. This ability to regenerate has been examined by Duncker (1905), Morgan (1900, 1902), Nussbaum and Sidoriak (1900), Mazza (1890) and Broussonet (1786). The following experiments were made to test the effect of cutting off the fins close to the body. The regenerative powers of the operculum, the lower jaw, the scales and the lens were also tested.

The various sets of experiments described below were tried on different fish, no one fish having more than one fin removed, except in the case of pectorals and pelvics which were removed from the same fish, the right pectoral and left pelvic being always selected for convenience.

I wish to express my thanks to Prof. T. H. Morgan, who kindly suggested this problem and under whose direction it was completed. My thanks are also due to the staff of the New York Aquarium, and to Dr. F. B. Sumner, director of the U. S. Fisheries Laboratory at Wood's Hole, Mass., who kindly placed the necessary tanks and fish at my disposal.

Dorsal Fin.

On October 10, 1905, eighteen fish had the dorsal fin cut off as close to the body as possible. These were examined from time to time until December 29, 1905, when due to various causes, principally fungal growth, the number had become reduced to thirteen. All of these with one exception were regenerating a new fin. The excepted case was kept under observation three months longer but at the end of that time, twenty-six weeks after operation, it had developed only a small

pointed knob in the middle of the old scar. The remaining twelve were not kept after December 29 (twelve weeks after operation). In all of these the regeneration was proceeding rapidly at this time, some having the new fin half completed.

On February 16, 1906, a second set of twenty-five fish had the dorsal fin cut off close to the body. These regenerated more rapidly than the first set and in five weeks' time, twenty-one of these which still remained, were regenerating along the entire length of the cut surface, in many cases the new growth being one eighth of an inch in height. Three weeks later, fifteen only were still living; the increase in height of the fins was distinctly visible in this short time, though more so in some individuals than in others. Three weeks later (at the time of writing) and twenty-three weeks after operation, fourteen were still alive. The new fins in some cases were regenerating evenly and possessed the normal number of fin rays (eleven). But in other cases, the new growth appeared along part of the cut surface only. An examination of the anatomy of the fin and the exact direction of the cut reveals the cause of this irregularity. Usually in trimming the fin close to the body, the dermal fin-rays are severed a short distance distal to their articulation with the bony supporting rays, beneath the surface. Sometimes, however, if the flesh at the sides of the fin is pressed down by the scissors and the fin at the same time held firmly, the cut will pass, in some places, through the enlarged proximal part of the dermal fin rays and regeneration in consequence will be much slower, or may not take place at all in parts so affected. The cut may even at times remove the dermal rays completely. These facts, I think, will account for cases in which regeneration is irregular, *i. e.*, when the new structure does not contain the normal number of fin-rays. Thus complete or nearly complete removal of the fin-rays prevents regeneration of the fin.

Anal Fin.

The anal fins of eighteen fish were cut off close to the body on October 25, 1905. The wounds healed up rather quickly but showed no signs of regeneration until two months later, when of the thirteen fish remaining, five showed slight signs of

new growth. Six weeks later the regeneration was fairly started in five fish which still survived. These five were examined one week later, *i. e.*, sixteen weeks after removal of the fin and it was found that three showed slight regeneration while the other two had a new fin about half the size of the original one. In these last, the fin was normal in shape and position, though small, and the rays parallel to one another as in the old fin. Ten weeks later, twenty-one weeks after removal of original fin, the regeneration was almost complete, the new fin having the normal number of fin-rays and being scarcely distinguishable from ordinary anal fins.

Here as in the case of the dorsal fin, the cut usually passes a short distance distal to the articulation of the dermal with the bony fin rays. But it may pass a little further in through the articulations if not carefully made, as the body is soft in the region of this fin. A cut of this kind results, as might be expected, in no regeneration. The cut may, however, pass through the articulations of some rays and the shafts of others; then the new growth takes place from the cut ends of the latter only, producing an irregular structure with less than the normal number of fin-rays.

The external prolongation of the oviduct in the females, which passes down the anterior end of the fin is, of course, severed with the fin. It regenerates, as might be expected, only when the anterior fin-ray which supports it, regenerates, not otherwise.

Caudal Fin.

On October 5, 1905, the caudal fins were removed from eighteen fish, the cut being made in a curve so as to remove the entire fin, without at the same time cutting off any of the tail vertebræ proper. This operation is rather severe; there is usually some bleeding and the edges do not close over the wound very readily. In consequence the number of fish lost is usually considerable. After twelve weeks, five fish only remained, all showing regeneration of the fin to some extent. Two weeks later the regeneration was still proceeding slowly but somewhat irregularly, the new fin-rays being bent downward, forming a considerable angle with the original horizontal direc-

tion of the rays, and the rays themselves were fewer in number than in normal fins. This result was due to very close cutting; entire rays were removed in some places, and in others, where stumps remained an alteration in direction of these stumps occurred in some way when the wound healed over. In one case the new fin grew straight out in the normal direction but at different rates of growth in different parts so that it presented a finger-like appearance. This abnormality was probably due to a jagged cut, the fin-rays being disarticulated in some places while in others, the stumps were left in situ. In still another fish no regeneration took place at all although the wound healed over smoothly. This fish was kept under observation four months. Here the cut probably either disarticulated the rays or left too small a stump for regrowth to take place.

On January 9, 1906, the tails of twenty-four fish were cut off in such a way as to remove a portion of the body of the fish, including several vertebræ. Obviously this operation was extremely severe. These fish when returned to water were unable to assume a horizontal position but remained head downward at the top of the tank. Three days later eleven were still living. These latter had succeeded finally in reaching the bottom of the tank and some were swimming about in a nearly normal position. At this time, January 12, another set of twenty-four fish, cut as described above, were put into the same tank with the eleven just mentioned. Three days later only seventeen were left of the entire number, thirty-five. This number gradually decreased until the entire lot died within a month's time.

Another set of twenty-four were cut in the same way but it was found impossible to keep them alive. The wounds remained open and the flesh rotted away from the sides of the vertebræ.

In the above sets, various sizes of fish were used. In still another set ten *small* fish were employed and kept in shallow dishes in the laboratory in order that they might assume a horizontal position. Within a month all of these had died with one exception. This might have been due to unfavorable conditions in part, though I have kept fish living in these shallow dishes over three months. In the exception noted, the wound had healed over smoothly and a very small white knob of tissue ap-

peared over the cut end of the spinal cord. This fish was kept under observation for three months but during this time the white knob did not increase perceptibly in size. As far as external examination could determine no regeneration of bone took place.

Pectoral and Pelvic Fins.

On October 25, 1905, twenty-four fish had their right pectoral and left pelvic fins removed. The cuts were made as close to the body as possible. In three months all died but three. These showed no regeneration of the pectoral fin but in one case a slight indication of a regenerating pelvic. Two weeks later two of these were still living, both showing the regenerating pelvics well under way and a well-defined indication of regenerating pectorals. Unfortunately the two fish died a few days later. Another set of twelve fish were cut in the same way but all died within a month, showing no signs of regeneration.

A third set of twenty-five fish cut as above, were started on February 16, 1906. Nine weeks later eighteen were still living; five of these showed regenerating pelvics and one, an indication of regenerating pectoral; the rest no regeneration of either fin. Two weeks later (at the time of writing) seventeen were living. Two of these showed regenerating pectorals distinctly, in the shape of a small knob of tissue which no doubt contained two or three regenerating rays, since the fish moved the structure to and fro like a fin. Eight of this same lot were regenerating their missing pelvic fins, the new structure in some cases being one eighth of an inch long.

An examination of the direction of the cut in the removal of the pectoral fins shows that in most cases all the dermal fin rays are removed entire, *i. e.*, disarticulated. Occasionally, however, the cut does not completely remove the uppermost one or two rays. These rays extend inward a little further than the others and so their proximal stumps may remain in situ and give rise to little pointed structures as described above, but never to a complete fin. In the case of the pelvic fins, it is not possible as a rule to cut them so close in; thus it more frequently happens that stumps of the rays are left, sometimes a few, sometimes all. Regeneration takes place from the cut ends of these only.

From the foregoing facts, the lack of regeneration of the paired fins in so many cases is seen to be due to complete removal of the dermal fin rays. These results fall in line with those described above for the unpaired fins.

Operculum.

On January 15, 1906, a set of six fish had a piece of the operculum removed, in order to determine whether the actual bony structures of the fish would regenerate. The piece removed was about one eighth of an inch broad and half an inch long, and was taken from the lower posterior border. Six weeks later two of these fish remained, but showed no signs of regeneration. A few days later, these also died. One fish with operculum cut as above was kept in a dish in the laboratory for four months, but at the end of this time showed no signs of regeneration.

The same experiment was tried with two sets of fifteen fish each at Wood's Hole. These fish were kept from June 20, 1906, to August 25, 1906, but at the end of this period showed no signs of regeneration.

Fish operated on in the above manner are sometimes difficult to keep alive since the gills are at first exposed to attacks of parasites and the nibbling of other fish. In a short time, however, the branchiostegal membrane spreads over and partly closes the opening.

Lower Jaw.

On March 23, 1906, pieces about one quarter of an inch long, were removed from the right-hand side of the lower jaws of thirty-six fish. Six weeks later, twenty-eight were still living. The jaws had grown again to such an extent that some of them could scarcely be distinguished from normal ones. Whether new bone took the place of that removed, was not accurately determined, though microscopic examination of sections of three of these new structures seemed to indicate that cartilage at least had regenerated.

Attempts were also made to remove larger portions of the jaw, or the entire jaw, but these always resulted in the death of the fish even if the wound healed. This may have been due to

the difficulty the fish experienced in grasping and holding its food.

Scales.

On June 20, 1906, the scales were removed from definite areas of a number of fish. This operation was performed in such a way as to injure as little as possible the underlying dermis, each scale being separately pulled out with a pair of fine forceps. Three sets of fifteen fish each were used in this experiment, each set of fish having the scales removed from a different part of the body. In three weeks time the new scales were coming in rapidly, some of them being one third the normal size, though very thin and soft. Two weeks later, the new scales were still only one-half to three quarters the length of a fully grown scale, though many were as broad. It appears then, that regenerating scales grow faster in breadth than in length. At this period or a little earlier the characteristic dotted pigment appears. At first the dots are comparatively few in number but continue to increase until the pigmentation, normal, for the position of the scale is attained. Thus each scale, from whatever region it is taken, is replaced by a new one pigmented exactly like the original. The fish in this experiment were kept ten weeks in all and at the end of that period the regenerated scales were practically normal in size, thickness and color though sometimes slightly irregular in shape.

Lens.

On June 21, 1906, the lens was removed from one eye of a number of fish. The operation was performed by slitting the cornea with a sharp scalpel and then gently pressing the eyeball with forceps until the lens popped out. In all thirty-six fish were used which were divided into two sets of eighteen each. In one of these sets the iris was slit in addition. These fish were kept until August 29, about ten weeks, when eleven fish remained in one set and nine in the other. When the lens is squeezed out some of the vitreous humor goes with it and after the operation the eyeball appears flattened. However the cornea heals over very quickly and the eye soon assumes its regular contour, differing only from normal apparently, in a slight dilation of the pupil.

The lost vitreous humor is apparently made good in some way. An examination of the eye at the end of the experiment (after ten weeks) revealed not the slightest indication of a new lens. Regeneration of the lens in *Triton* and *Salamandra*, has been obtained by Colucci ('91), Wolff ('95) and Fischel ('98). These results cannot then be extended to the teleost, *Fundulus heteroclitus*. However, it still remains to be shown whether the regenerative powers of the eye are not better developed in other species of teleosts.

Summary and Conclusions.

The general conclusions to be drawn from the foregoing experiments are the following:

1. In *Fundulus heteroclitus* the dorsal, caudal, anal, pectoral and pelvic fins will regenerate even when cut off close to the body, provided the proximal ends of the dermal fin-rays remain in situ. No regeneration takes place except when the stumps are present, and of sufficient size.

2. The operculum does not regenerate.

3. The lower jaw completes itself if a piece is removed.

4. The scales, if carefully removed, are quickly replaced by others.

5. The eye does not regenerate a new lens.

The results on the regeneration of fins support in general Broussonet's earlier work and that of subsequent writers. The ability to regenerate a portion of the jaw has been found by Spallanzani (1768) and others in the salamander. The lack of ability to regenerate a new lens in *Fundulus*, however is peculiar, since the salamander, an animal of a higher order, possesses it to a marked degree. It would be interesting in this connection to test the regenerative powers of the eyes of elasmobranches and ganoids, as being more generalized types than the teleosts.

In regard to Broussonet's statement that those fins which are the most useful, regenerate the quickest, it seems more probable that the conditions of the cut, as described for each experiment above, are the factors which determine the rate of regeneration, given equal conditions of temperature, food, vitality, etc. Indeed it is difficult to estimate the relative rate of growth, for in any

one set of fish kept in the same tank and having the same fins removed, the differences in the rate of renewal are so great that some of the new fins may have completed half their growth when others are just beginning to show definite regeneration. Again, the caudal fin though obviously the most important in many fish does not regenerate, perceptibly, faster than the others. Finally, leaving aside the caudal it is difficult to say which fin is the most important. The observations which Osburn ('06) has recently made on the functions of fins seem to indicate that the pectorals are more useful than the dorsals and anals while my own results show that the former regenerate much slower. Broussonet was probably led to his statement concerning the relative rate of regeneration in different fins by his acceptance of the idea of regeneration as an adaptation. *A priori*, the caudal fin would regenerate, the most rapidly, on the hypothesis, since it is the most useful. The facts as I have tried to show, do not bear this out.

At this point the question naturally arises as to the relation between regeneration and liability to injury. It is true that the fins, extending out from the body as they do, are subjected to injury through brushing against obstructions, attacks of predatory animals, etc.; therefore, one might say, they acquired the power to regenerate as an adaptation to these conditions. But why, on the adaptation hypothesis, should the jaw regenerate so readily, when it is extremely improbable that this structure would ever be lost in a natural state? From the lack of regenerative power in the operculum and eye no conclusions can be drawn for or against this hypothesis, since these structures are not especially liable to injury under normal conditions. The power to regenerate scales, however, seems truly adaptive.

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October 6, 1906.

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